Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of chlorite dismutase: a detoxifying enzyme producing molecular oxygen

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Chlorite dismutase, a homotetrameric haem-based protein, is one of the key enzymes of (per) chlorate-reducing bacteria. It is highly active (>2 kU mg(-1)) in reducing the toxic compound chlorite to the innocuous chloride anion and molecular oxygen. Chlorite itself is produced as the intermediate product of (per) chlorate reduction. The chlorite dismutase gene in Azospira oryzae strain GR-1 employing degenerate primers has been identified and the active enzyme was subsequently overexpressed in Escherichia coli. Chlorite dismutase was purified, proven to be active and crystallized using sitting drops with PEG 2000 MME, KSCN and ammonium sulfate as precipitants. The crystals belonged to space group P2(1)2(1)2 and were most likely to contain six subunits in the asymmetric unit. The refined unit-cell parameters were a = 164.46, b = 169.34, c = 60.79 angstrom. The crystals diffracted X-rays to 2.1 angstrom resolution on a synchrotronradiation source and a three-wavelength MAD data set has been collected. Determination of the chlorite dismutase structure will provide insights into the active site of the enzyme, for which no structures are currently available.
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